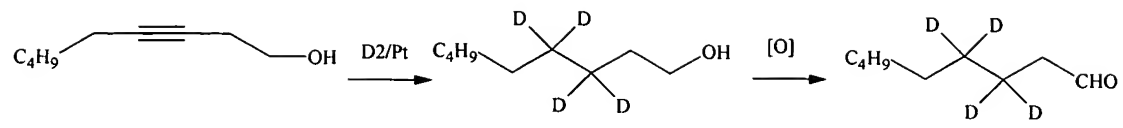


Figure 2 : Synthesis of nonanal-d4

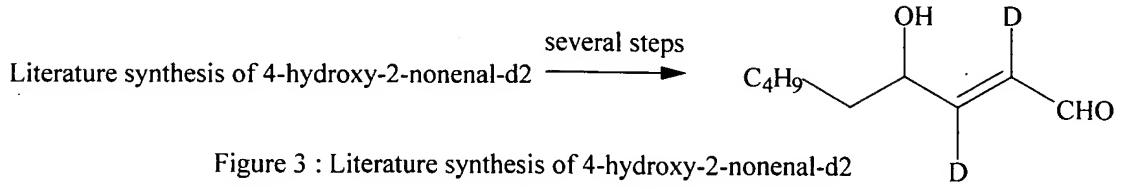


Figure 3 : Literature synthesis of 4-hydroxy-2-nonenal-d2

If MS analysis of the same aldehydes is performed using our method, only one step synthesis is be required. The products are the deuterated oximes:

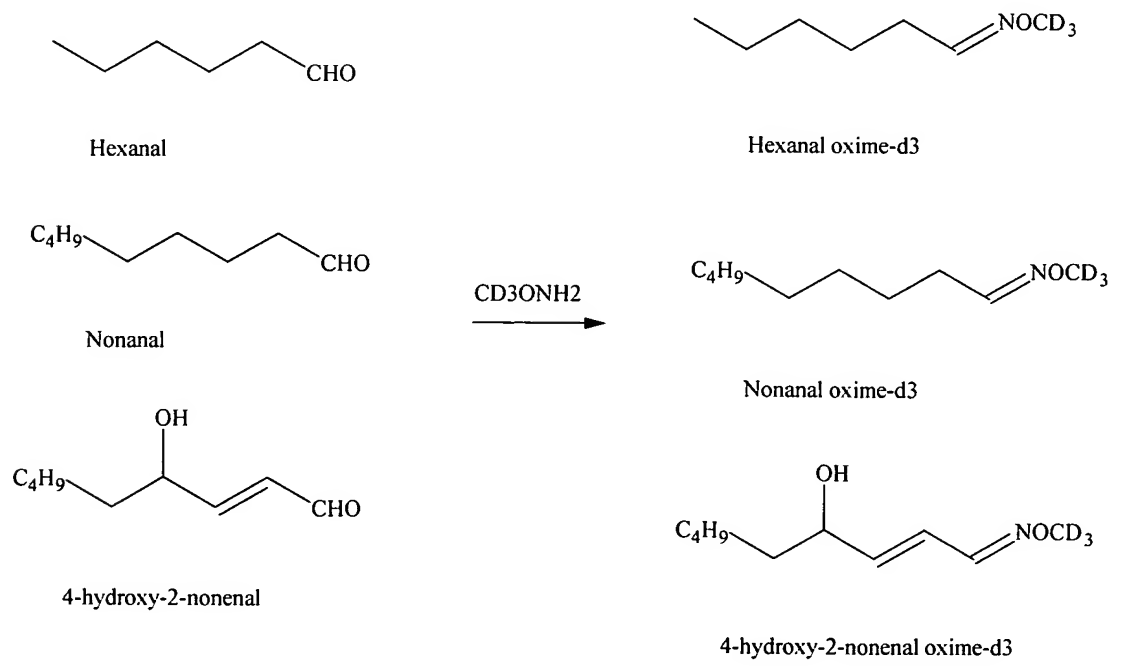


Figure 4 : Synthesis of deuterated oximes-d3

As shown in the above figures, the stable isotope labeled oxime internal standards were synthesized from deuterated aldehydes and ketones in Bruenner et al's prior arts while the same internal standards were synthesized from non deuterated aldehydes and ketones in the present invention. Moreover, the present invention requires the synthesis of the non-labeled oxime equivalents in the presence of the stable isotope labeled oxime internal standards whereas the oxime conversion is 100% quantitative. The present

invention further requires there is no conversion of said stable isotope labeled oxime internal standards to their corresponding non-labeled oxime. These specific requirements were not required nor mentioned in Bruenner et al's prior arts. As a result, a person having ordinary skill in the art to which said subject matter pertains is not expected to know or perform these specific requirements. In other words, the arts of the present invention would not be obvious to a person having ordinary skill in the art.

Prior arts of Ludeman illustrated the use of deuterated oximes as stable isotope labeled internal standards for the MS analysis of 4-hydroxycyclophosphamide and aldophosphamide. The synthesis of each deuterated aldehyde and deuterated oxime is also a multi step synthesis.

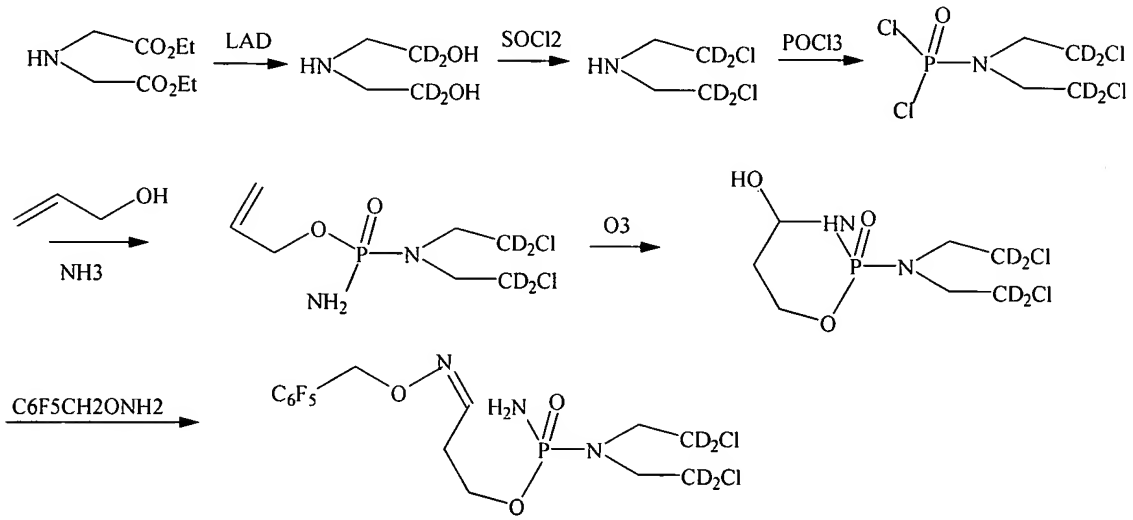


Figure 5 : synthesis of Ludeman's 4-hydroxycyclophosphamide-d4 and its oxime-d4

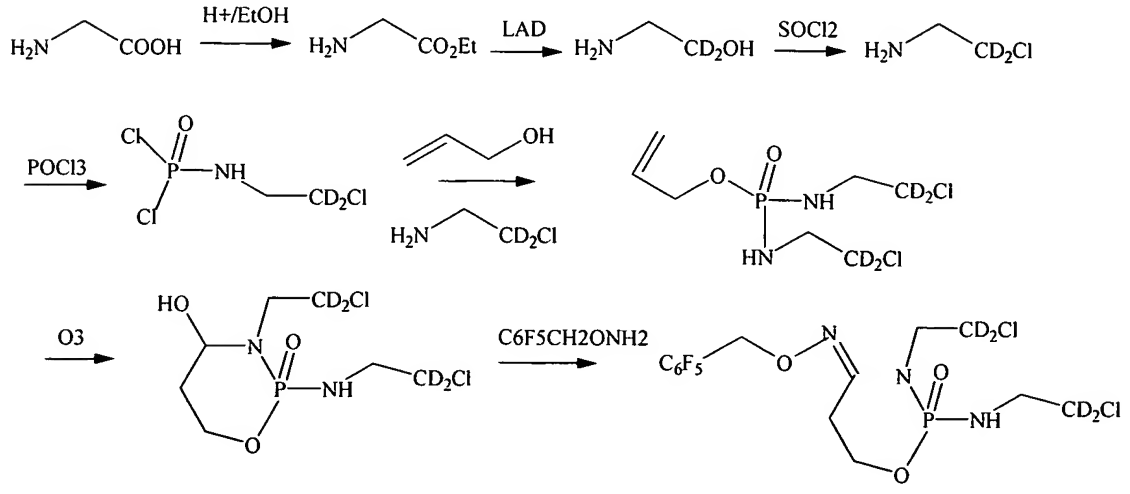
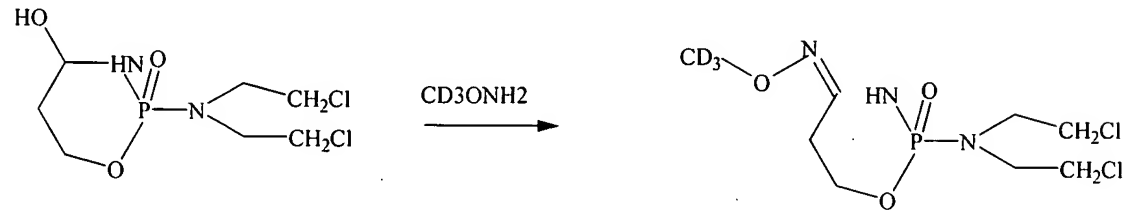


Figure 6 : synthesis of Ludeman's aldophosphamide-d4 and its oxime-d4

If MS analysis of the same aldehydes is performed using our method, only one step synthesis is required . The starting materials are the authentic sample of the aldehyde metabolites. The products are the deuterated oximes :



4-Hydroxycyclophosphamide

4-Hydroxycyclophosphamide oxime-d3

Figure 7 : synthesis of 4-hydroxycyclophosphamide oxime-d3 from disclosed method

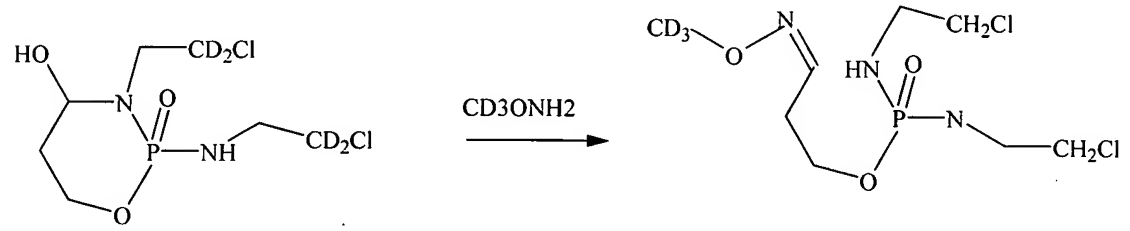
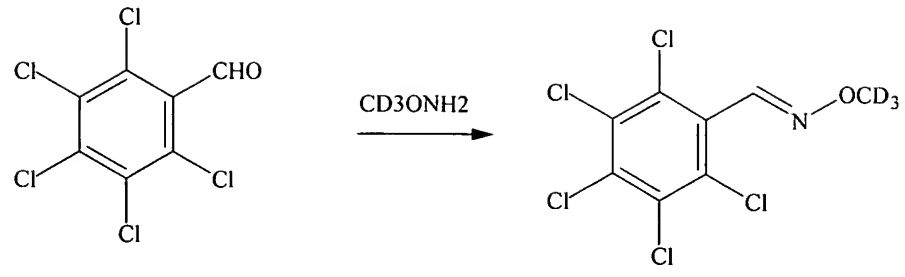


Figure 8 : synthesis of Aldphosphamide oxime-d3 from disclosed method

The simplicity and the economy in the synthesis of deuterated oxime internal standards are clearly recognized in our method compared to methods of Breunner and Ludeman. Our method eliminates the need for synthesis of deuterated aldehydes. Because our method provides a method to synthesize deuterated oximes from an authentic sample of the aldehydes and an available deuterated methoxylamine reagent, all deuterated oximes can be made the same way and possibly in the same reaction pot if desired.

b) Difference No. 2: If Bruenner's work or Ludeman's work required analysis of additional aldehydes and ketones, then independent synthesis of each deuterated aldehyde and deuterated ketone would be required. Not all syntheses of deuterated aldehydes or deuterated ketones are feasible. Our one step synthesis method of preparing deuterated oximes from aldehyde and ketone componds clearly illustrates the advantages over prior arts. Lengthy and costly syntheses of individual deuterated aldehydes and ketones are major drawback for many MS analyses of multiple aldehydes and ketones using deuterated internal standards.

c) Difference No.3 : For cases where synthesis of deuterated aldehydes and ketones are difficult, for example pentachlorobenzaldehyde, our method offers a solution for isotope dilution MS by making deuterated oximes using the same one step synthesis.



Pentachlorobenzaldehyde

Pentachlorobenzaldehyde oxime-d3

Figure 9 : Synthesis of pentachlorobenzaldehyde oxime-d3

The issue we address is not an improvement of isotope dilution MS as noted in the work of Kingston. We address the solution for synthesis of stable isotope labeled aldehydes and ketones in their MS analysis as oxime derivatives. Formation of oxime derivatives from aldehydes and ketones has been recognized as a very efficient reaction at room temperature and even in an aqueous environment as in the case of analysis of aqueous samples. Kingstons' work never implied, inferred, or addressed the MS analysis of aldehydes and ketones by oxime derivatives.

For an person with an ordinary skill in the art, it is not obvious from the works of Breunner and Ludeman that deuterated oxime internal standards can be prepared from an authentic sample of those aldehydes and ketones and labeled alkoxylamines. Instead, Breunner et al and Ludeman et al synthesized deuterated aldehydes and deuterated ketones before converting them to deuterated oxime internal standards. The fact that i) our method provides an alternative way to synthesize labeled oxime internal standards that are useable in MS analysis of aldehydes and ketones without requiring the availability of labeled aldehydes and ketones; and ii) that our one step synthesis of labeled oxime internal standards can be applied to almost any compound having the aldehyde and ketone functionality, we ask that you consider these facts the proof of nonobviousness and allow claim 1-15 regarding MS analysis of aldehydes and ketones as oxime derivatives.

4. In response to rejection of claims 16-20 and 22-30 as being unpatentable in view of prior art of Zurek et al. for determining acetaldehyde in tobacco smoke by isotope dilution LCMS, we wish to point out the differences between prior art and our method.

   a) Zurek's work did not use stable isotope labeled hydrazine in the synthesis of labeled hydrazone internal standard. Instead, a non-labeled hydrazone MNBDH was allowed to react with C13 labeled acetaldehyde to form labeled acetaldehyde hydrazone internal standard. It was possible that C13 acetaldehyde was available but labeled MNBDH was not. Zurek's analysis only determined the concentration of acetaldehyde. But if the concentration of other cited aldehydes such as formaldehyde, propanal, butanal, acrolein, benzaldehyde, and p-tolylaldehyde were required to be determined, then the availability of their stable isotope labeled version would be required. These may not be commercially available, or else their syntheses must be needed. Using our method stable isotope labeled hydrazone internal standards can be available from labeled methylhydrazine or benzylhydrazine and a sample of the authentic aldehydes.

b) Zurek's work required hydrazine reagent MNBDH which has UV chromophore for HPLC detection. Our method does not require hydrazines with a chromophore for MS analysis.

5. In response to rejection of claim 21 as being unpatentable in view of prior art of Zurek et al for determining of aldehydes and ketones in air samples by LCMS using 2,4-dinitro-3,4,6-trideuterophenylhydrazine (DNPH), we would like to point out the differences between prior art and our method.
   a) Zurek's work required a hydrazine with a chromophore such as the nitro-functional group. Therefore a special synthesis of deuterated DNP reagent was performed.
   b) Zurek's work provided quantification of aldehydes and ketones in gas samples while our method applies to aqueous samples that require isolation of labeled hydrazone internal standards and non-labeled hydrazones by an aqueous extraction. For aqueous samples, particularly aqueous biological samples such as blood, plasma, body fluid etc, macromolecules such as protein and nucleic acids are major sources of ion suppression in MS analysis. Analytes and internal standards are required to be isolated by means such as liquid liquid extraction or solid phase extraction to be free of macromolecules and salts. The use of stable isotope labeled internal standards in MS analysis take into account the frequently low recoveries of both internal standards and analytes from aqueous solution. As long as their recoveries are the same (because they are isotope derivatives), their low extraction recoveries do not alter the signal ratios of analyte to internal standard. These ratios are plotted against analyte concentration for the construction of the calibration curve in isotope dilution MS. In other word, differences in recovery of internal standards and analytes are the major cause of non-linearity in MS analysis. That is why stable isotope internal standards is the solution for this non-linearity in the MS analysis of aqueous solutions. When aqueous extraction is not required, then there is no need to use stable isotope labeled internal standard in MS analysis.

      For a person with an ordinary skill in the art, it is clear that the use of stable isotope labeled internal standards in MS analysis take advantages of the fact that both analytes and their respective labeled internal standard will be have the same chemically, as in the case of chemical derivatization in gas chromatography-mass spectrometry (GCMS) analysis, and physically, as in the case of aqueous extraction either as liquid liquid extraction or solid phase extraction. Their derivatization reactions in GCMS analysis do not have to be complete or quantitative and their extraction recoveries do not have to be quantitative either. This sets the difference in MS analysis using labeled internal standards versus MS analysis using non labeled analogs. It is not clear in Zurek's work that deuterated hydrazone internal standards were required in the MS analysis of aldehydes in car smoke samples in which no aqueous extraction was required. HPLC separation as you mentioned was not regarded as isolation by aqueous extraction. The fact that i) our method provides an alternative way to synthesize labeled hydrazone internal standards that are useable in MS analysis of aldehydes and ketones without requiring the availability of labeled aldehydes and ketones; and ii) that our one step synthesis of labeled hydrazone internal standards can be applied to almost any compound having the aldehyde and ketone functionality, and iii) that our method can be applied to MS analysis of aqueous samples that require isolation of both labeled hydrazone internal standards and non-labeled hydrazone by aqueous extraction, we ask that you consider these facts the proof of nonobviousness and allow claim 16-30 regarding MS analysis of aldehydes and ketones as hydrazone derivatives.

In summary, besides the numerous material and significant differences between the present invention and the prior arts of Bruenner et al, Ludeman et al and Zurek et al mentioned above, the present invention further requires the synthesis of the non-labeled oxime and hydrazone equivalents in the presence of the stable isotope labeled oxime and hydrazone internal standards whereas the oxime and hydrazone formation is 100% quantitative. The present invention further requires there is no conversion of said stable isotope labeled oxime and hydrazone internal standards to their corresponding non-labeled oximes and hydrazones. These specific requirements were not required nor mentioned in Bruenner et al, Ludeman et al and Zurek et al's prior arts. As a result, a person having ordinary skill in the art to which said subject matter pertains is not expected to know or perform these specific requirements. In other words, the arts of the present invention would not be obvious to a person having ordinary skill in the art. We ask that claims 1-30 be allowed based on these arguments.

Kind regards,

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